Bradyrhizobium lablabi sp. nov., isolated from effective nodules of Lablab purpureus and Arachis hypogaea

Yue Li Chang,1 Jing Yu Wang,1 En Tao Wang,2 Hong Can Liu,3 Xin Hua Sui1 and Wen Xin Chen1

1State Key Laboratory for Agro-Biotechnology, Key Laboratory of Agro-Microbial Resource and Application, Ministry of Agriculture, College of Biological Sciences, China Agricultural University, Beijing, 100193, PR China
2Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, 11340 México D. F., Mexico
3Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, PR China

Five strains isolated from root nodules of Lablab purpureus and Arachis hypogaea grown in the Anhui and Sichuan provinces of China were classified as members of the genus Bradyrhizobium. These strains had identical 16S rRNA gene sequences which shared 99.48% similarity with the most closely related strains of Bradyrhizobium jicamae PAC68T, Bradyrhizobium pachyrhizi PAC48T and Bradyrhizobium elkanii USDA 76T, respectively. A study using a polyphasic approach, including 16S rRNA gene RFLP, IGS-RFLP, BOX-PCR, comparative sequence analysis of the 16S–23S rRNA intergenic spacer (IGS) and the recA, atpD and glnII genes, DNA–DNA hybridization and phenotypic tests, showed that the five strains clustered into a coherent group that differentiated them from all recognized species of the genus Bradyrhizobium. Sequencing of nifH and nodC genes and cross-nodulation tests showed that the representative strains CCBAU 23086T, CCBAU 23160 and CCBAU 61434, isolated from different plants, had identical nifH and nodC gene sequences and were all able to nodulate Lablab purpureus, Arachis hypogaea and Vigna unguiculata. Based upon these results, the name Bradyrhizobium lablabi sp. nov. is proposed for this novel species and strain CCBAU 23086T (=LMG 25572T=HAMB1 3052T) is designated as the type strain. The DNA G+C mol% is 60.14 (Tm).

Arachis hypogaea L. (peanut or groundnut) is an important legume crop that provides food and edible oil for direct human consumption. It plays a significant part in the economy of many countries in the world (El-Akhal et al., 2006). Most rhizobial isolates nodulating peanut belong to the genus Bradyrhizobium, such as Bradyrhizobium japonicum (Chen et al., 2003; El-Akhal et al., 2009; Taurian et al., 2006), although some other effective fast-growing rhizobia have also been described (El-Akhal et al., 2008; Taurian et al., 2006).

Lablab purpureus originated in Africa and has been widely distributed in many tropical and subtropical countries where it has become naturalized. In China, L. purpureus is grown as an annual or a short-lived perennial in different regions. In these areas, the seeds and immature pods are used for human consumption while the herbage is used as a green manure (Murphy & Colucci, 1999). To date, only a few rhizobial strains isolated from L. purpureus nodules have been studied and most of these have been identified as members of the genus Bradyrhizobium (Morrison et al., 1986), except for the broad host range strain NGR 234 (Trinick, 1980).

During a study of rhizobia nodulating A. hypogaea and L. purpureus grown in southern China, 73 isolates (of a
Bradyrhizobium lablabi sp. nov.

Total of 93) were characterized and classified in the genus *Bradyrhizobium* based on 16S rRNA gene RFLP, 16S–23S rRNA intergenic spacer (IGS) RFLP and sequence analyses of *rrs*, IGS, *atpD*, *recA*, *nodC* and *nifH* genes (unpublished data). Within these bradyrhizobia, five isolates (Table 1) exhibited distinct characteristics and differed from recognized species of the genus *Bradyrhizobium*. For 16S rRNA gene RFLP, the gene was amplified with primers P1 and P6 by the PCR protocol of *Tan et al.* (1997) from DNA extracted from each strain by a routine method (*Terefelew et al.*, 2001). The amplified fragments were digested with *MspI*, *HinDIII*, *Alul* and *HaeIII* as specified by the manufacturer. The five strains had identical patterns and were defined as the same rRNA type, which grouped together with *Bradyrhizobium jicamae* PAC68T, *Bradyrhizobium pachyrhizi* PAC48T and *Bradyrhizobium elkanii* USDA 76T (see Supplementary Fig. S1 in IJSEM Online) in the cluster analysis using the DICE coefficient and the UPGMA method.

In IGS-RFLP fingerprints, the DNA fragments were amplified with the primers FGPS1490 and FGPL132 (Laguerre *et al.*, 1996) as described by *Kwon et al.* (2005). The fragments were digested with *MspI*, *HindIII* and *HaeIII*. As found in the 16S rRNA gene analysis, the five strains shared the same pattern, which showed the highest similarity (65%) with *B. jicamae* PAC68T and *B. pachyrhizi* PAC48T (Supplementary Fig. S2), indicating that the five strains might represent a novel species.

BOX-PCR fingerprinting is a powerful tool to estimate the genomic diversity of bacteria and to identify strains (de *Bruijn, 1992; Nick & Lindstrom, 1994; Nick et al., 1999*). In the present study, BOX-PCR was performed to characterize the five strains by using the primer BOXAIR (5'-CTACGGCAAGGCGACGCTGACG-3') and the procedure of *Versalovic et al.* (1994). The amplified products were subjected to electrophoresis in 1.5% (w/v) agarose gels stained with ethidium bromide. The electrophoretic patterns were analysed by the Gelcompar II program and a UPGMA dendrogram was constructed. Three BOX-PCR fingerprints were obtained, indicating that the strains were not members of the same clone. The strains formed a cluster at 75% similarity (see Supplementary Fig. S3), which was greater than the similarities obtained among the three recognized species of the genus *Bradyrhizobium*.

Currently, 16S rRNA gene sequencing is used as the principal method to define a bacterial genus, but it is not sufficiently sensitive to distinguish between closely related species. Due to this limitation, analysis of the IGS region has become a useful tool for determining relatedness among closely related bacteria, including bradyrhizobial strains (*Willems et al.*, 2001, 2003). In addition to the IGS region, several other phylogenetic markers, such as the *atpD*, *recA* and *glnII* genes, have been proposed for species and genospecies descriptions within the genus *Bradyrhizobium* (*Vinuesa et al.*, 2005). In accordance with previous studies and the RFLP results in the present study, three strains (CCBAU 23086T, CCBAU 23160 and CCBAU 61434) representing different BOX types and different host origins were chosen for sequence analyses of the IGS and 16S rRNA, *atpD*, *recA*, *glnII*, *nodC* and *nifH* genes, as well as for DNA–DNA hybridization.

The 16S rRNA gene and IGS region of representative strains were amplified in the same manner as in the RFLP analysis. The *atpD*, *recA* and *glnII* genes were amplified and sequenced by using the method of *Vinuesa et al.* (2005), with primer sets atpD255F/atpD782R, *recA*41F/recA640R and glnII12F/glnII689R, respectively. The amplification and sequencing of partial *nodC* and *nifH* genes was performed with the primers nodC540/nodC1160 (*Sarita et al.*, 2005) and nifH1F/nifH1R (*Laguerre et al.*, 2001), respectively. All the amplified fragments were directly sequenced as described by *Hurek et al.* (1997). The sequences were aligned with those of related species of the genus *Bradyrhizobium* using the CLUSTAL W program in the MEGA 4.0 software package (*Kumar et al.*, 1994, 2008). Aligned sequences were analysed by using MEGA 4.0 software to produce a Jukes–Cantor distance (Jukes & Cantor, 1969) and to construct an optimal unrooted tree using the neighbour-joining (NJ) (*Laguerre et al.*, 1996; *Saitou & Nei, 1987*) method. Bootstrap analysis was based on 1000 replications (*Felsenstein, 1985*). Maximum-likelihood (ML) trees were constructed as unrooted trees using PhyML 3.0 (*Guindon & Gascuel, 2003*). The robustness of the ML topologies was inferred by non-parametric bootstrap tests based on 100 data.

---

Table 1. Strains used in this study and relevant information

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host plant</th>
<th>Geographical origin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. lablabi</em> sp. nov.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCBA 23086T</td>
<td><em>L. purpureus</em></td>
<td>Anhui, China</td>
<td>This study</td>
</tr>
<tr>
<td>CCBA 23160</td>
<td><em>A. hypogaea</em></td>
<td>Anhui, China</td>
<td>This study</td>
</tr>
<tr>
<td>CCBA 61434</td>
<td><em>L. purpureus</em></td>
<td>Sichuan, China</td>
<td>This study</td>
</tr>
<tr>
<td>CCBA 61428</td>
<td><em>L. purpureus</em></td>
<td>Sichuan, China</td>
<td>This study</td>
</tr>
<tr>
<td>CCBA 61430</td>
<td><em>L. purpureus</em></td>
<td>Sichuan, China</td>
<td>This study</td>
</tr>
<tr>
<td><em>B. pachyrhizi</em> PAC48T</td>
<td><em>Glycine max</em></td>
<td>USA</td>
<td><em>Kuykendall et al.</em>, 1992</td>
</tr>
<tr>
<td><em>B. jicamae</em> PAC68T</td>
<td><em>Pachyrhizus erosus</em></td>
<td>Costa Rica</td>
<td>Ramirez-Bahena <em>et al.</em>, 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honduras</td>
<td>Ramirez-Bahena <em>et al.</em>, 2009</td>
</tr>
</tbody>
</table>
sequences and were closely related to (Fig. 1), the three strains tested had identical gene
sequences of the three genes is shown in Fig. 2 and the ML
tree is available as Supplementary Fig. S6. The strains of the
novel group were closely related to
B. jicamae PAC68\textsuperscript{T},
B. pachyrhizi PAC48\textsuperscript{T} and
B. elkanii USDA 76\textsuperscript{T}, respectively. Since
Willems et al. (2003) have hypothesized that two bra-
dyizobial strains with IGS region sequence similarity
<95.5 % usually belong to separate genospecies, our data
suggested that the novel group represented a novel
genospecies of the genus Bradyrhizobium.

The results of the phylogenetic analyses of the three
housekeeping genes with the NJ and ML methods were
similar. The NJ tree constructed using the combined
sequences of the three genes is shown in Fig. 2 and the ML
tree is available as Supplementary Fig. S6. The strains of the
novel group were closely related to B. jicamae PAC68\textsuperscript{T},
B. pachyrhizi PAC48\textsuperscript{T} and B. elkanii USDA 76\textsuperscript{T}, which
confirmed the relationships obtained in analyses of the
16S rRNA gene. The sequence similarities of the
atpD, recA and glnII genes were 98.4–100 % among the three
representative strains of the novel group and were <95.6 %
between the novel group and reference strains of related
species (see Supplementary Table S1). The small differences
between strains CCBAU 23086\textsuperscript{T} and CCBAU 23160 in the
atpD gene sequence (98.98 % similarity) demonstrated that
the strains were not a clone, although both shared the same
BOX patterns.

Symbiotic (nif and nod) genes do not offer taxonomic
information because they are located in easily interchange-
able elements (plasmids or in symbiotic islands), but they
are required for the successful establishment of the highly
specific symbiosis between rhizobia and legumes. There-
fore, comparisons of these symbiotic genes may reveal the
host ranges of rhizobia. For this reason, the nifH and nodC
genes are commonly analysed for the description of novel
rhizobial species. The results of the phylogenetic analysis of
these genes in the present study showed that the rhizobia
isolated from L. purpureus and A. hypogaea had identical
nifH and nodC gene sequences, implying that they could
have the same host spectrum.

In the nifH gene phylogenetic tree (Supplementary Fig. S7),
the closest relatives were strain CCBAU 83335 nodulating
Sophora alopecuroides, RST89 nodulating Retama spha-
ecarpa and CCBAU 05065 nodulating Vicia pseudorobus,
with 96.5 %, 96.5 % and 96.3 % sequence similarities,
respectively. The nifH sequence similarities between the
novel group and B. jicamae PAC68\textsuperscript{T}, B. pachyrhizi PAC48\textsuperscript{T}
and B. elkanii USDA 76\textsuperscript{T} were 92.2 %, 88.0 % and 88.5 %,
respectively. When the nodC genes were considered, the
similarities between the novel group and recognized species
were <86 %, indicating an independent evolutionary
history.

As a standard method for species delineation (Graham
et al., 1991; Wayne et al., 1987), DNA–DNA hybridization
was performed between a representative strain, CCBAU
23086\textsuperscript{T}, and reference strains for the novel group (CCBAU
23160 and CCBAU 61434) and for related species
(B. jicamae PAC68\textsuperscript{T}, B. pachyrhizi PAC48\textsuperscript{T} and
B. elkanii USDA 76\textsuperscript{T}) using Marmur’s method for total DNA
isolation (Marmur, 1961) and the renaturation-rate
technology described previously by De Ley et al. (1970).

---

**Fig. 1.** Neighbour-joining tree reconstructed from 16S rRNA gene sequences showing the phylogenetic relationships of strain CCBAU 23086\textsuperscript{T}. Bootstrap values >50 % are indicated at nodes. The sequence of Rhizobium leguminosarum USDA 2370\textsuperscript{T} was used as an outgroup. Bar, 1 % nucleotide substitutions.
All experiments were performed three times and the mean DNA–DNA relatedness is presented in Supplementary Table S1. The DNA–DNA relatedness of strain CCBAU 23086T with strains CCBAU 23160 and CCBAU 61434 was 100 % and 92.74 % (Supplementary Table S1), respectively. The results of DNA–DNA hybridization between strain CCBAU 23086T and the related type strains B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.

To discern and describe the novel genomic species, the cellular fatty acid profile of strain CCBAU 23086T was assayed together with those of B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.

To discern and describe the novel genomic species, the cellular fatty acid profile of strain CCBAU 23086T was assayed together with those of B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.

To discern and describe the novel genomic species, the cellular fatty acid profile of strain CCBAU 23086T was assayed together with those of B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.

To discern and describe the novel genomic species, the cellular fatty acid profile of strain CCBAU 23086T was assayed together with those of B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.

To discern and describe the novel genomic species, the cellular fatty acid profile of strain CCBAU 23086T was assayed together with those of B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.

To discern and describe the novel genomic species, the cellular fatty acid profile of strain CCBAU 23086T was assayed together with those of B. jicamae PAC68T, B. pachyrhizi PAC48T and B. elkanii USDA 76T (Supplementary Table S1) indicated that strain CCBAU 23086T showed significantly low relatedness values, in the range of 20.09–31.74 %. These values were lower than the threshold value of 70 % DNA–DNA relatedness that is generally accepted as the threshold for the definition of a species (Wayne et al., 1987).

The G + C content of the DNA was measured by the thermal denaturation method of De Ley et al. (1970) using Escherichia coli K-12 as a standard. The DNA G + C content of the three strains was 60.14–62.85 mol% (Tm), which was within the range expected for recognized members of the genus Bradyrhizobium.
host range is an important feature for the description of novel rhizobial species (Graham et al., 1991). In the present study, cross nodulation tests performed in vermiculite moistened with N-free solution (Vincent, 1970) indicated that strains CCBAU 23086^T, CCBAU 23160 and CCBAU 61434 could nodulate L. purpureus, A. hypogaea and Vigna unguiculata, but not Glycine max, Trifolium repens, Lotus corniculatus, Vigna radiata, Pisum sativum or Medicago sativa under laboratory conditions.

According to all the results obtained in this study, it is concluded that the five new strains represent a novel species in the genus Bradyrhizobium. The name Bradyrhizobium lablabi sp. nov. is proposed for this taxon with strain CCBAU 23086^T designated as the type strain.

**Description of Bradyrhizobium lablabi sp. nov.**

Bradyrhizobium lablabi (lab’la.bi. N.L. gen. n. lablabi of Lablab referring to the fact that the bacterium was isolated from a root nodule of Lablab purpureus).

Cells are Gram-negative, aerobic, non-spore-forming rods. Colonies on YMA medium are circular, convex and translucent and have a diameter of 1 mm after incubation for 7–10 days at 28 °C. The generation time is 10–12 h in YM broth. The pH range for growth is 5–10, with optimum growth at pH 7.0. Growth occurs between 10 °C and 37 °C, with optimum growth at 28 °C. Does not grow in the presence of 1 % (w/v) NaCl. In addition to the carbon sources listed in Table 2, the type strain is also able to utilize D-galactose, sodium DL-malate, D-ribose, sodium D-glucuronate, d-arabinose, hippuric acid, sodium succinate,

### Table 2. Distinctive features of B. lablabi sp. nov. and its closest relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation time (h) in YM broth</td>
<td>10–12</td>
<td>10–12</td>
<td>10–12</td>
<td>6–7*</td>
<td>7–8*</td>
<td>&gt;6*</td>
</tr>
<tr>
<td>Utilization of sole carbon sources:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipic acid</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Melezitose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sodium formate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of sole nitrogen sources:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glutamic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resistance to (µg ml⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin (50)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Streptomycin sulfate (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tetracycline HCl (150)</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythromycin (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythromycin (50)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gentamicin sulfate (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chloramphenicol (50)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in/at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 % (w/v) NaCl</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>pH 5.0</td>
<td>w</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>pH 10.0</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>37 °C</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Data from Ramírez-Bahena et al. (2009).
†The data from this study were not consistent with those reported previously.
D-sorbitol, sorbose, soluble starch, tartaric acid, D-xylene, L-proline and calcium gluconate as sole carbon sources. Does not utilize adic acid, D-amygdalin, lactose, D-mannose, L-methionine, melibiose, raffinose, succrose, syringic acid, L-arginine, DL-asparagine, glycine, L-glycine, dextrin or dulcitol as a sole carbon source. Is able to grow on DL-alanine, L-arginine, hypoxanthine, L-isoleucine, L-phenylalanine, L-valine, L-aspartic acid and L-lysine as sole nitrogen sources. No growth with glycine, L-glutamic acid or L-methionine as sole nitrogen sources. The type strain is resistant to (µg mL⁻¹), chloramphenicol (5), kanamycin (5), neomycin sulfate (5), tetracycline hydrochloride (150) and gentamicin (5). Detailed distinctive features and the fatty acid content are shown in Table 2 and Supplementary Table S2. Strains can be distinguished by their housekeeping gene sequences and by DNA–DNA hybridization (Supplementary Table S1).

The type strain, CCBAU 23086T (=LMG 25572T=HAMBI 3052T), was isolated from effective nodules of *L. purpureus* in Anhui province, China. The DNA G+C content of the type strain is 60.14 mol%. Additional strains are CCBAU 23160 and CCBAU 61434.

Acknowledgements

We thank Dr Encarna Vela´zquez (Departamento de MicroBiología y Genética, Universidad de Salamanca, Spain) for supplying the *B. pachyrhizi* PAC68T and *Gene´tica, Universidad de Salamanca, Spain*) for supplying the *J Basic Microbiol* in Moroccan soils.


